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Fate of Ingested Chrysotile Asbestos Fiber in the Newborn Baboon

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16. ABSTRACT

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Many methodological problems were encountered in preparing tissues for evaluation by electron microscopy. After developing a satisfactory preparation technique, tissue samples of kidney cortex, kidney medulla, spleen, and liver were analyzed. Definitive data are presented which demonstrate the recovery of chrysotile asbestos from kidney cortex tissue. Data concerning the kidney medulla, spleen and liver tissues must be considered preliminary as more definitive work is in progress concerning these and other tissues.

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by

William H. Hallenbeck
Kusum Patel-Mandlik
School of Public Health
University of Illinois
Chicago, Illinois 60680

Order No. CA-7-3159-J

Project Officer

James R. Millette
Exposure Evaluation Branch
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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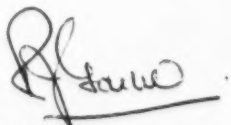
This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The U. S. Environmental Protection Agency was created in response to increasing public concern about the dangers of pollution to the health and welfare of the American people and their environment. The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines.

The Health Effects Research Laboratory was established to provide sound health effects data in support of the regulatory activities of the EPA. Evaluating man's exposure to environmental health hazards is a key segment in developing such a data bank. Studies of exposure require indentification, characterization, and quantification of physical, chemical, and biological agents found in the environment. In addition, exposure assessment involves the determination of conditions that cause agents to be released into the environment, the study of the routes and pathways to man, and research into the body's ability to prevent the entrance of environmental hazards.

This report presents the results of a study to determine if ingested asbestos fibers can penetrate the gastrointestinal tract and be transported to other tissues in the body. An understanding of how the body handles asbestos is important in determining the potential health effects of asbestos in drinking water.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

The objective of this research was to determine if orally administered chrysotile asbestos fibers can penetrate through the gastrointestinal tract of the neonate baboon and be recovered in selected tissues. A neonate baboon was given a cumulative dose of 3×10^{13} chrysotile fibers per kilogram. At the end of a 9-day feeding period test and control animals were sacrificed. The following tissues were collected: kidney, liver, spleen, heart, lymph nodes, urine, blood, and gastrointestinal tract.

Many methodological problems were encountered in preparing tissues for evaluation by electron microscopy. After developing a satisfactory preparation technique, tissue samples of kidney cortex, kidney medulla, spleen, and liver were analyzed. Definitive data are presented which demonstrate the recovery of chrysotile asbestos from kidney cortex tissue. Data concerning the kidney medulla, spleen and liver tissues must be considered preliminary as more definitive work is in progress concerning these and other tissues.

This report was submitted in fulfillment of Contract No. WOO112 by the School of Public Health, University of Illinois Medical Center under the sponsorship of the U. S. Environmental Protection Agency. This reports covers the period September 8, 1977 to September 15, 1978 and work was completed September 15, 1978.

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SECTION 1

INTRODUCTION

Asbestos is being ingested by the general population with the primary route of exposure being drinking water.[1-6] An important environmental health question concerns whether or not chronic exposure to ingested asbestos can cause cancer at one or more sites. Recent studies indicate that ingested asbestos fibers can penetrate through the gastrointestinal tract and migrate to various organs.[7] Fiber penetration is a matter of concern because translocated fibers may induce cancer at susceptible sites anywhere in the body. Possible sites include the lung, gastrointestinal tract, plura, peritoneum, and other sites.[8-17]

The main objective of the present study was to determine if fibers could be recovered from various tissues of a neonate baboon which had been administered chrysotile asbestos in milk suspension. This protocol represents a departure from all previous animal ingestion experiments which used a rat or mouse mode. With the exception of the study by Cunningham, et al,[7] previous animal ingestion studies have provided inconclusive answers to the question of fiber penetration of the gut wall. See Hallenbeck and Hesse[18] for a review of animal ingestion experiments which were published before 1976. In light of Cook and Olson's (personal communication) work with humans, it became important to test the hypothesis of fiber penetration in a controlled animal experiment which employed a species closely related to man. Hence the baboon was selected primarily for phylogenetic reasons. In addition, the neonate baboon can be taken from the mother at birth and readily bottle-fed a known concentration of asbestos fibers suspended in milk formula.

SECTION 2

CONCLUSIONS

The findings of this study strongly indicate that orally administered chrysotile asbestos fibers penetrate through the gut wall of the neonate baboon and that migration to the kidney cortex occurs. Also it appears that fibers with a wide range of lengths penetrate through the gastrointestinal tract of the neonate baboon and that no selection mechanism is operative. Further work is in progress which involves the in-depth analysis of kidney medulla, liver, spleen, and other tissues from the same test and control animals sacrificed in the present study.

SECTION 3

MATERIALS AND METHODS

GENERAL PROCEDURES

Two neonate baboons (Papio anubis), born exactly 8 days apart, were used as control and test animals. Each animal was maintained in an Isolette infant incubator under conditions of controlled temperature, humidity and air supply. The test animal weighed 920 grams and was bottle fed with a suspension of UICC Canadian chrysotile in milk formula. See Table 1 for the frequency distribution of UICC fiber lengths. The suspension was prepared by swirling 10 mg of asbestos in 500 ml of distilled water which had been previously filtered through a 0.1 μm Millipore membrane. It was mixed 1:1 with concentrated milk formula (Similac, Ross Laboratories) prior to feeding to give 10 mg of chrysotile per liter. Assuming 10^6 fibers per nanogram, the feeding suspension contained 10^{13} fibers per liter (f/ℓ). A total of 2.8 liters was provided ad libitum over a period of 9 days beginning 24 hours after birth. Therefore, the total dose for the test animal was 3.0×10^{13} F/kg. The control animal weighed 961 g and was bottle-fed with a one to one mixture of concentrated milk formula and distilled, filtered water; 2.6 liters were consumed over 9 days. Both animals were sacrificed at the end of the 10-day trial. The following tissues were collected from each animal after perfusion and bleeding: gastrointestinal tract, spleen, liver, kidney, urine, lymph nodes, blood, and heart.

Tissues preparation and analysis technique

1. Comparable weights of control and test tissues were dried in a vacuum oven at 90°C until weights became constant.

2. Dried tissue was ashed in a low temperature plasma asher at 80 watts for 3 hours.

3. The resultant ash was suspended in 10 ml of 1% acetic acid and ultrasonicated at 50 kHz for 1 minute to obtain dispersion without breakage. Treatment with acid improved the removal of organics in the subsequent ashing step. A check using reference UICC Canadian chrysotile indicated that acid treatment did not disturb the morphology or diffraction pattern under transmission electron microscopy (TEM).

4. The suspension was filtered through a 0.1 μm Nucleopore membrane and the latter was ashed in the plasma unit at 80 watts for 3 hours. The residue was suspended in 5 ml of filtered (0.2 μm Fluoropore) acetone and sonicated for one minute.

TABLE 1. FREQUENCY DISTRIBUTION OF FIBER LENGTHS OF U.I.C.C. CANADIAN CHRYSOTILE AND CHRYSOTILE FIBERS FOUND IN THE KIDNEY CORTEX

Length range (in μm)	U.I.C.C.[20] %	Kidney cortex %
0.2-0.5	30.6	37.6
0.5-1	33.4	26.6
1-2	19.8	23.0
2-5	13.2	5.5
5-10	1.76	3.7
10-25	0.93	2.7
25-50	0.24	0.9
50-100	0.07	0
100-200	0.00	0

5. 6 μl were immediately withdrawn and deposited on a carbon and Formvar coated TEM grid. Rapid drying was effected by the use of an infrared lamp (250 watts at 5 inches from the grid).

6. Chrysotile fibers were identified by morphology, and randomly selected area electron diffraction (SAED) patterns were obtained with a Philips 300 TEM. Grids were scanned at a magnification of 19,000 and in all cases an equal number of test and control grid holes were scanned.

Calculation of concentration and detection limit

The concentration of fibers per mg day weight of tissue was calculated using the following equation:

$$\text{Fibers/mg dry weight of tissue} = \frac{\bar{F} \times A_1 \times V_1}{A_2 \times V_2 \times M} = \frac{7 \times 10^5 \bar{F}}{M}$$

where F = average number of fibers per grid square

$A_1 = 0.07\ cm^2$ = area of a 200 mesh TEM grid

$A_2 = 8 \times 10^{-5}\ cm^2$ = area of one grid square

$V_1 = 5$ ml = volume of sample suspension

V_2 = 0.006 ml = volume of suspension deposited on grid

M = mass of tissue prepared (mg dry weight)

The detection limit of this analytical technique can be calculated by substituting $\overline{F}$ = 0.1 and a particular dry weight, say M = 100 mg, in the above equation. This substitution yields a detection limit of 700 fibers per mg (dry weight) of tissue.

SECTION 4

RESULTS AND DISCUSSION

Kidney, spleen, and liver tissues from control and test neonates were prepared for TEM analysis. The preparation technique described above resulted in a uniform distribution of particles on the grid surface; this can be seen in the photomicrographs of the edge, intermediate, and center areas of one grid (see Figures 1-3). Also, analysis of blank data revealed no chrysotile fibers in 10 grid squares. See Table 2 for a description of blank data.

Preliminary screening of kidney, spleen and liver tissues (see Tables 3 and 4) indicated that the concentration of fibers in the kidney cortex was significantly greater than its control ($P = 0.005$). The statistical evaluation of data such as that shown in Table 3 has been discussed by Hallenbeck, et al.[20]

In order to make a final analysis of the kidney cortex preparation, an additional 62 grid squares were scanned and the results are shown in Table 5. Final analysis of the kidney cortex preparations revealed a statistically significant excess of fibers in the test preparation as compared to control preparation ($P < 0.005$). See Tables 1 and 6 for detailed information concerning the length, diameter, and aspect ratio of fibers found in the kidney cortex. It can be seen in Table 1 that there is very little difference in the frequency distributions of fiber lengths of UICC Canadian chrysotile and fibers found in the kidney cortex. Hence, there appears to be no fiber selection process occurring in the gastrointestinal tract of the neonate baboon. Additional data is being collected on kidney medulla, spleen, liver, and other tissues in order that final analyses can be made concerning the level of chrysotile fibers in these tissues.

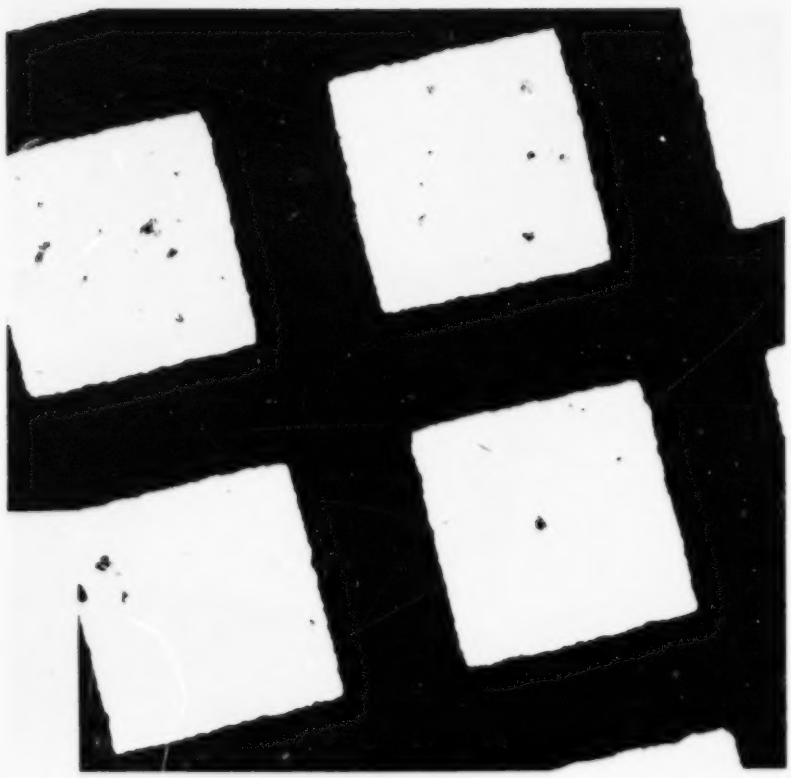

Figure 1. Photomicrograph (600x) of a preparation of neonate baboon spleen taken near the edge of a TEM grid. Note the uniform distribution of particulates. The same preparation was used in Figures 1-3.

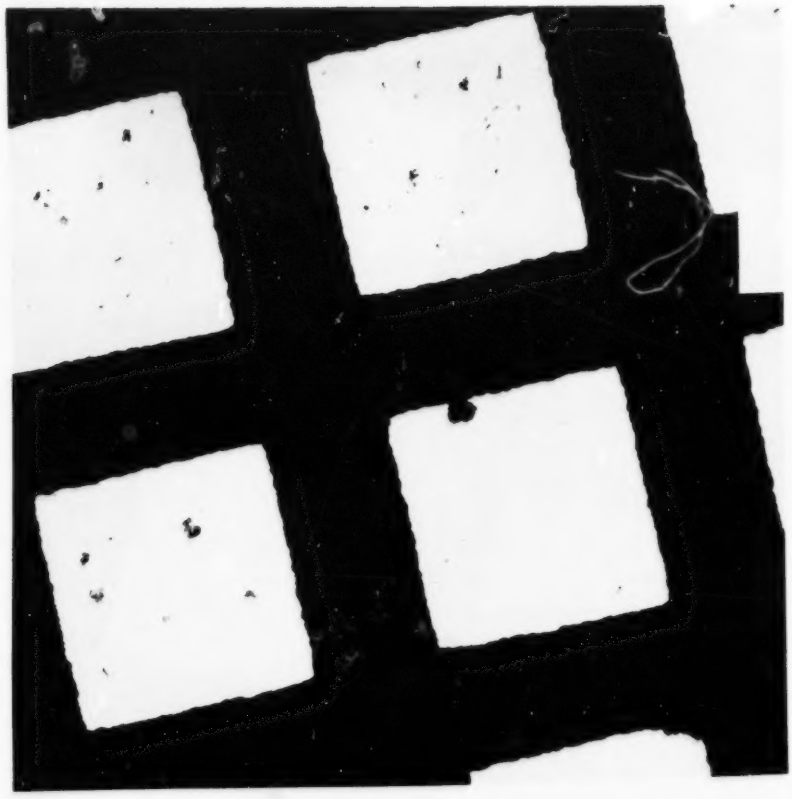

Figure 2. Photomicrograph (600x) of a preparation of neonate baboon spleen taken in the center area of a TEM grid. Note the uniform distribution of particulates. The same preparation was used in Figures 1-3.

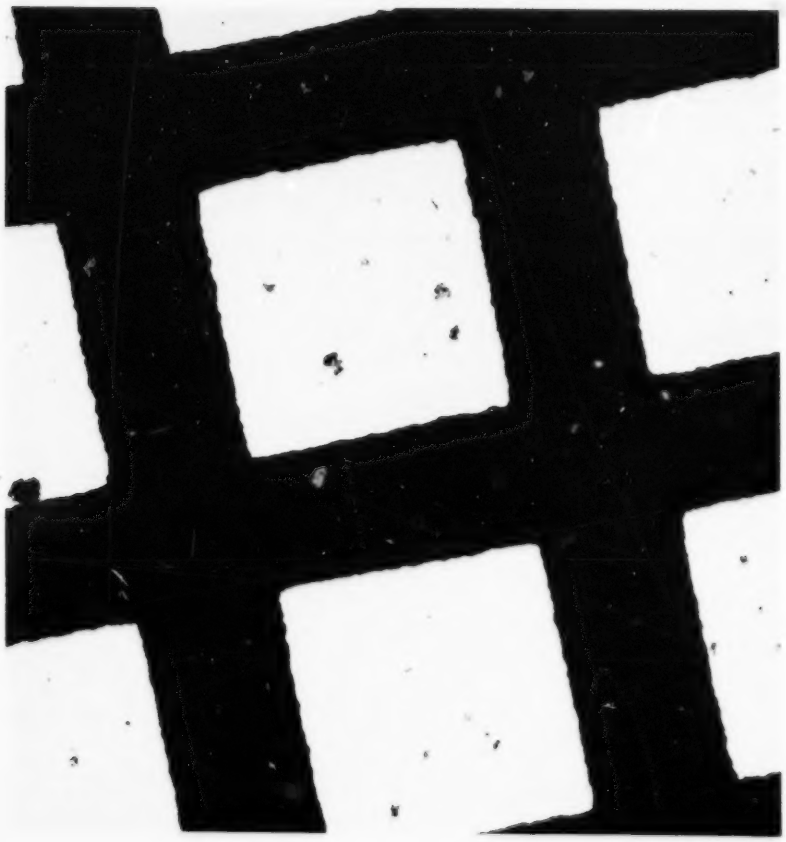

Figure 3. Photomicrograph (800x) of a preparation of neonate baboon spleen taken in the intermediate zone of a TEM grid. Note the uniform distribution of particulates. The same preparation was used in Figures 1-3.

TABLE 2. BLANK DATA

Grid square number	Blank 1*	Blank 2*
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
Total fibers	0	0

* Blank 1 was prepared by placing 6 µl of acetone (previously filtered through a 0.2 µm Fluoropore membrane) on a TEM grid with a carbon coated Formvar substrate. Blank 2 was prepared by placing 6 µl of an acetone suspension (5 ml) of an ashed Nucleopore membrane (0.1 µm) on a TEM grid with a carbon coated Formvar substrate.

TABLE 3. RESULTS OF PRELIMINARY SCANNING OF SELECTED TISSUES OF NEONATE BABOONS

Grid square number	Number of chrysotile fibers							
	Kidney cortex		Kidney medulla		Liver		Spleen	
	C[a]	T[a]	C	T	C	T	C	T
1	0	0	0	2	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	2	0	0	0	0
6	0	1	0	0	0	0	0	0
7	0	6	0	0	0	0	0	1[b]
8	0	2	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	1	0	0	0	0	0	0
Total fibers	0	10[c]	0	4[d]	0	0	0	1

[a] T = test neonate; C = control neonate

[b] The single fiber observed in the spleen of the test neonate was composed of a bundle of 40-50 fibrils.

[c] P = 0.005

[d] P = 0.10.

TABLE 4. PRELIMINARY FIBER CONCENTRATIONS IN SELECTED TISSUES OF NEONATE BABOONS

	Kidney				Spleen		Liver	
	Medulla		Cortex					
	C[a]	T[a]	C	T	C	T	C	T
Dry weight (mg)	38	57	133	158	168	168	297	328
Chrysotile fibers/mg dry weight	BD[b]	2,456[d]	BD	4,430[c]	BD	417	BD	BD

[a] C = control neonate; T = test neonate, bottle-fed a total dose of 3.0×10^{13} chrysotile fibers/kg body weight in a suspension of milk formula.

[b] BD = below detection

[c] P = 0.005

[d] P = 0.10. Because of the low mass of medullar tissue 12 µl were deposited on the TEM grid rather than 6 µl.

TABLE 5. FINAL ANALYSIS OF KIDNEY CORTEX TISSUE OF NEONATE BABOON FOR CHRYSOTILE ASBESTOS

	Control Tissue	Test Tissue
No. of grid squares scanned	70	72
No. of chrysotile fibers found	0	119[a]
Dry weight (mg)	133	158
Chrysotile fibers/mg dry weight	BD[b]	7,322[a]

[a] $P < 0.005$

[b] BD = below detection

TABLE 6. LENGTH, DIAMETER, AND ASPECT RATION DATA FOR CHRYSOTILE FIBERS FOUND IN THE KIDNEY CORTEX OF THE NEONATE BABOON

	Length μm	Width μm	Aspect ratio
Average	1.7	0.04	39.5
Standard deviation	4.38	0.04	89.5
Maximum	35	0.34	800
Minimum	0.24	0.03	5
Median	0.5	0.03	16.7
Geometric mean	0.73	0.04	20.4

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